

Supplementary material

A gene expression atlas of a *bicoid*-depleted *Drosophila* embryo reveals canalization of cell fate

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Supplementary Figure Legends

- I. Fig. S1: Phenotypic spectrum of *bcd* RNAi cuticles resembles a published allelic series
 - A. Unhatched cuticles of embryos laid by *MTD-Gal4/UAS-shRNA-bcd* females display variable numbers of denticle bands. A representative individual from each class is shown. The filzkörper, a tail structure, is indicated with arrow heads. Scale bar 200 μ m.
 - B. An additional *UAS-shRNA-bcd* (GL01320) line gives a similar phenotype.
 - C. The maternal driver *mat-tub-Gal4* gives a similar phenotype with both *UAS-shRNA-bcd* lines.
 - D. To test viability, we arrayed 200 embryos on an agar plate and counted the number hatched after 48 hrs at 25°C. For *bcd* RNAi embryos, 0/200 and 0/200 embryos hatched; for embryos laid by *MTD-Gal4/UAS-shRNA-GFP* females 222/239 and 178/200 of embryos hatched (TRiP Toolbox stock 182) (Neumuller et al., 2012).

- II. Fig. S2: Mother age contributes strongly to the strength and variability of the *bcd* RNAi phenotype while temperature, *UAS-shRNA-bcd* line, maternal driver, zygotic *UAS-shRNA-bcd* construct copy number, and paternal genotype do not.
 - A. The severity of *bcd* phenotype increases as the flies ages. To quantify the effect of maternal age, we allowed flies to eclose for 48 hrs and collected cuticles from one cage of *MTD-Gal4/UAS-shRNA-bcd* females every few days and counted denticle bands. Old mothers are >10 days old and used to build the gene expression atlas. Counts: Day 2 n = 137, Day 6 n = 173, Day 8 n = 224, Day 11 n = 86, Day 14 n = 127, Day 16 n = 58.
 - B. qPCR indicates >90% knockdown in embryos laid by older mothers. Embryos were collected for 2 hrs. As a reference for WT *bcd* levels we averaged deltaCT counts from Days 3, 5, 8 and 12 of *MTD-Gal4/UAS-shRNA-GFP* embryos. We then subtracted this average number from the *bcd* RNAi deltaCT counts for Days 3, 5, 8, 10, and 14, converted the deltadeltaCT to percent of WT levels.
 - C. Paternal genotype does not meaningfully influence the *bcd* RNAi phenotype. *MTD-Gal4/UAS-shRNA-bcd* virgin females were crossed to males homozygous for *UAS-shRNA-bcd* or with males homozygous for a enhancer lacZ reporter (WT). Progeny from the first cross will have 1-2 copies of the *UAS-shRNA-bcd* construct (blue), while progeny from the second cross will have 0-1 copies of the *UAS-shRNA-bcd* construct (red). We could not detect a difference between the number of ventral denticle bands visible in each populations (p values from Kolmogorov-Smirnov test). Note that the effect of mother age is much greater than paternal genotype (compare left and right for Day 1 and Day 6 samples respectively). Although it is possible that shRNAs against *bcd* are zygotically expressed, they do not meaningfully contribute to the phenotype, consistent with the purely maternal effect of *bcd*. This cross also shows there is no detectable paternal effect. The absence of zygotic or paternal effects allowed us to integrate the reporter data into the gene expression atlas.
 - D. Two *UAS-shRNA-bcd* lines yield similar phenotypes. We tested two shRNA lines (GL00407 and GL01320) at 25°C and 29°C, taking samples on Day 4 and Day 10. For these crosses we used the *mat-tub-Gal4* maternal driver; this driver tends to give a more consistent phenotype than *MTD-Gal4*. ANOVA of temperature, UAS-line, and day reveal that each has a significant but small effect, less than one segment in all cases. For the *mat-tub-Gal4* driver, the distribution of phenotypes laid by young mothers approaches the steady state distribution seen for old mothers with *MTD-Gal4* more quickly. This result

is consistent with more uniform phenotypes for the *mat-tub-Gal4* driver with shRNAs against other genes (Staller et al., 2013). The distributions of embryos laid by old mothers of both genotypes are comparable. For future work depleting other maternal effect genes, we plan to use the *mat-tub-Gal4* driver.

- III. Fig. S3: *bcd* RNAi embryos have more cells and altered cell density patterns.
 - A. Average cell density maps of WT and *bcd* RNAi embryos. While the physical shape of the embryos remains asymmetric, the posterior density pattern is duplicated in the anterior of *bcd* RNAi embryos, like some mRNA patterns. Embryos from stage 5:51-100% (time points 5 and 6 in the gene expression atlases) are shown.
 - B. Histogram of cell counts in *bcd* RNAi embryos and WT (transgenic) embryos.
- IV. Fig. S4: The boundaries of the *eve* stripes move over stage 5 in *bcd* RNAi embryos.
 - A. Boundary positions calculated using inflection points of WT and *bcd* RNAi embryos. Stripe 4 and 5 appear in a handful of embryos in cohort 4, but not frequently enough to reliably quantify boundary position. In each plot, anterior is left, dorsal is top.
 - B. The widths of the *eve* stripes contract in *bcd* RNAi embryos. At T = 5 *eve* stripes 4-7 are approximately 1.7, .6, 1.4, and 1.3 cell widths wider in *bcd* RNAi than WT. At T = 6 *eve* stripes 4-7 are approximately 1.3, .6, 1.5, and .3 cell widths wider in *bcd* RNAi than WT. Data calculated from the side of the embryo.
- V. Fig. S5: The coefficient of variation of most the gap and *eve* stripe mRNA pattern widths are similar between WT (blue) and *bcd* RNAi (red). The exceptions are *Kr* and the anterior *eve* stripe, which are more variable in *bcd* RNAi embryos. The ventral region of the ectopic anterior *hb* pattern (DV strips 7-11) is very faint in *bcd* RNAi embryos, and our analysis script struggles to reliably find a boundary, so this analysis likely overstates the variability in this region. Pattern widths calculated with the inflection point. The ectopic anterior *hb* pattern in *bcd* RNAi is compared to the *hb* posterior pattern in WT.
- VI. Fig. S6: The locations of each transcription factor combination in each genotype. The “sum” of each class is shown at the bottom.
- VII. Fig. S7: Changing the ON/OFF threshold does not meaningfully change the conclusions of the combination analysis.
 - A. For a range of thresholds and most time points, all combinations present in *bcd* RNAi also present in WT.
 - B. When a combination is unique to *bcd* RNAi, this is generally because it was not detected in WT for that threshold and time point. At high thresholds, overlap between adjacent patterns (*Kr* and *kni* or *kni* and *gt*) are not detected in WT. At T=4, the adjacent *hb*, *hkb* and *tll* patterns do not overlap enough to be detected in WT at most thresholds, but this combination is found at adjacent time points, so it is not a true new combination. T=6 is explained in the main text.
 - C. Line traces of *Kr*, *hkb*, and *tll* at T=6 in WT and *bcd* RNAi. Anterior-posterior position is on the x-axis and expression level is on the y-axis. The high levels of background in the WT *hkb* pattern may confound the combination analysis.

VIII. Fig. S8: Thresholds, stain hapten, and mother age do meaningfully influence the fraction of cells that express both *eve* and *ftz*.

- A. The hapten (DIG or DNP) of the *eve* mRNA probe does not bias this analysis. *ftz* is always in the opposite channel.
- B. A schematic shows the effects of varying the ON/OFF threshold for either *eve* or *ftz*. To find the threshold we create a histogram of the expression level of each gene separately and identify the peak of the OFF cell population (mode). For our normal threshold, we add one standard deviation. We varied the threshold to be 0.6, 0.8, 1 and 1.2 standard deviations.
- C. C-F) Regardless of the ON/OFF threshold used, the fraction of cells expressing both *eve* and *ftz* (double ON cells) decreases over time. The mixed mom *eve* DNP embryos (magenta) were collected from cages that had a wide range of mother ages, before we started collecting from aged cages. We included these data because they have a very high quality stain. These data indicate that mother ages does not have a meaningful effect on this analysis. In Fig. 6 the red and magenta data are combined. In all plots, error bars are the standard error of the mean.

IX. Fig. S9: In *bcd* RNAi the *eve2* enhancer reporter has limited expression of stripe 7 in the posterior and some duplicated stripe 7 in the anterior.

- A. A maximum intensity projection of the *eve2 lacZ* stain.
- B. DNA (green), *ftz* (blue), and *lacZ* (red).

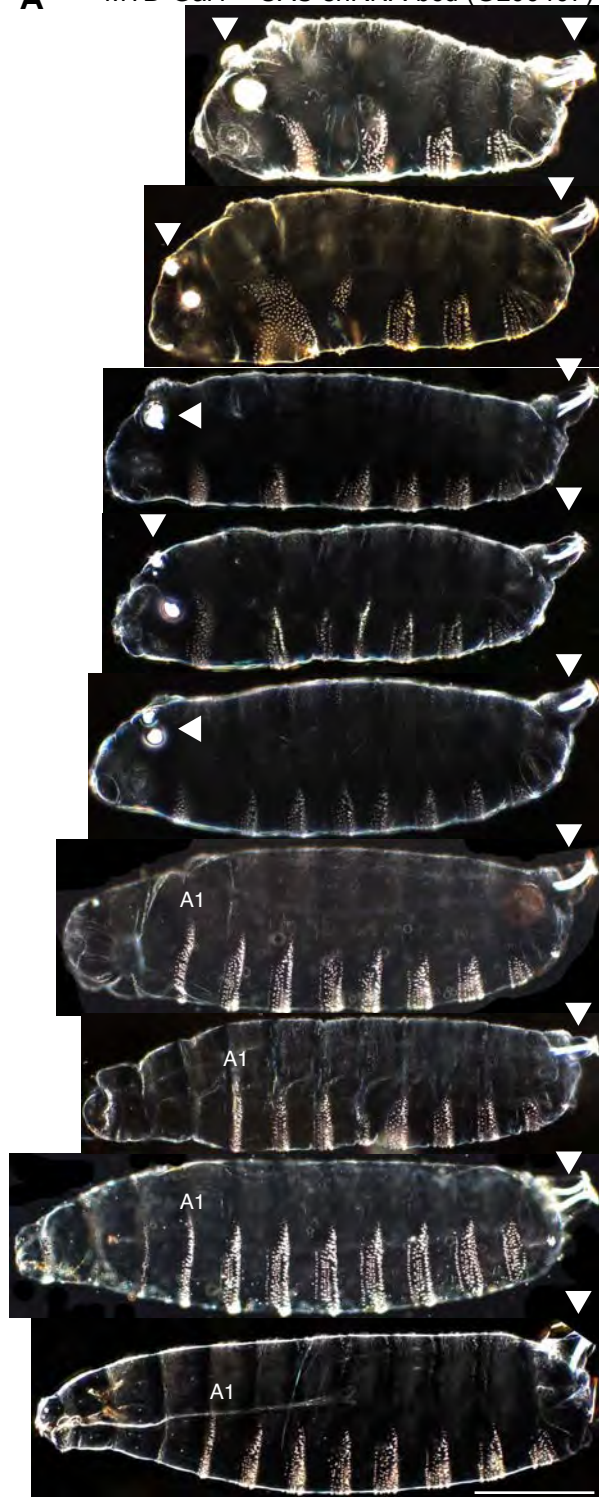
Table S1: The numbers of individual embryos for each gene at each time point included in the *bcd* RNAi gene expression atlas

Table S2: The standard deviations of each gene in the *bcd* RNAi gene expression atlas. Data from the WT atlas is included for comparison (Fowlkes et al., 2008).

Table S3: ON/OFF thresholds used in the combination analysis in Fig. 5, S7.

Table S4: Sequences of the enhancer *lacZ* reporter constructs included in the *bcd* RNAi atlas.

A MTD-Gal4>>UAS-shRNA-bcd (GL00407)



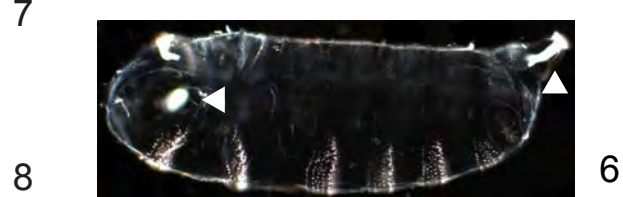
B MTD-Gal4 >> UAS-shRNA-bcd (GL01320)



5 C mat-tub-Gal4 >> UAS-shRNA-bcd (GL0407) 29C



mat-tub-Gal4 >> UAS-shRNA-bcd (GL01320)



WT

Figure S1

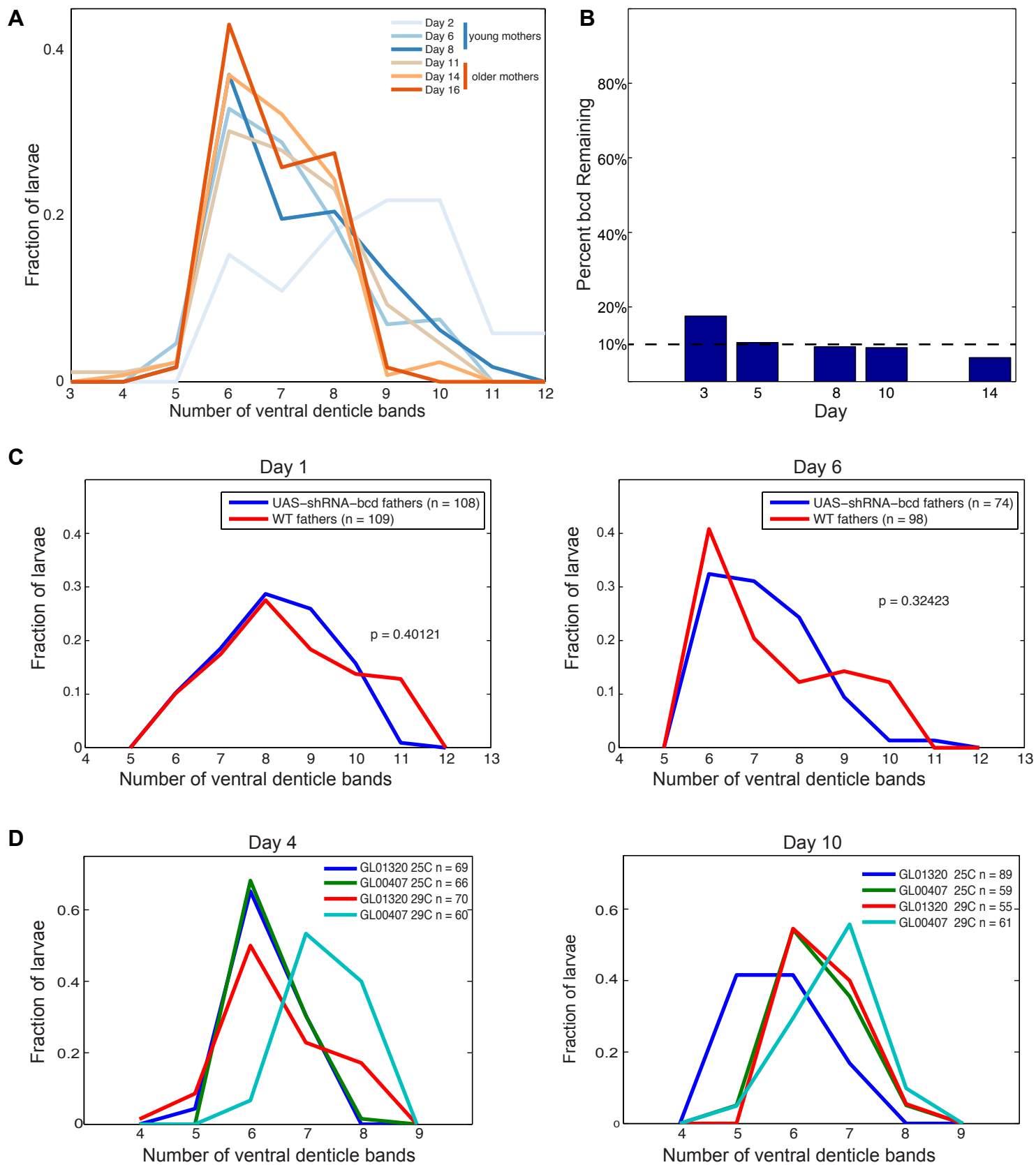


Figure S2

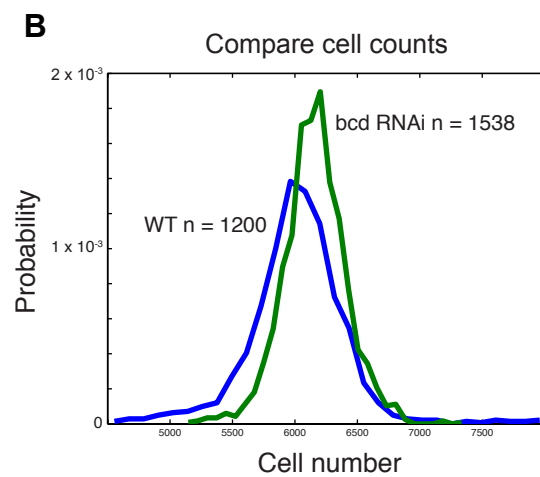
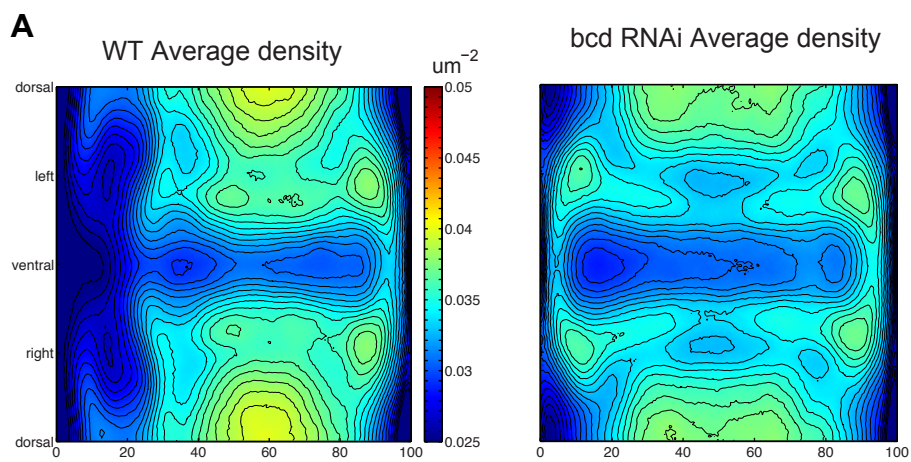


Figure S3

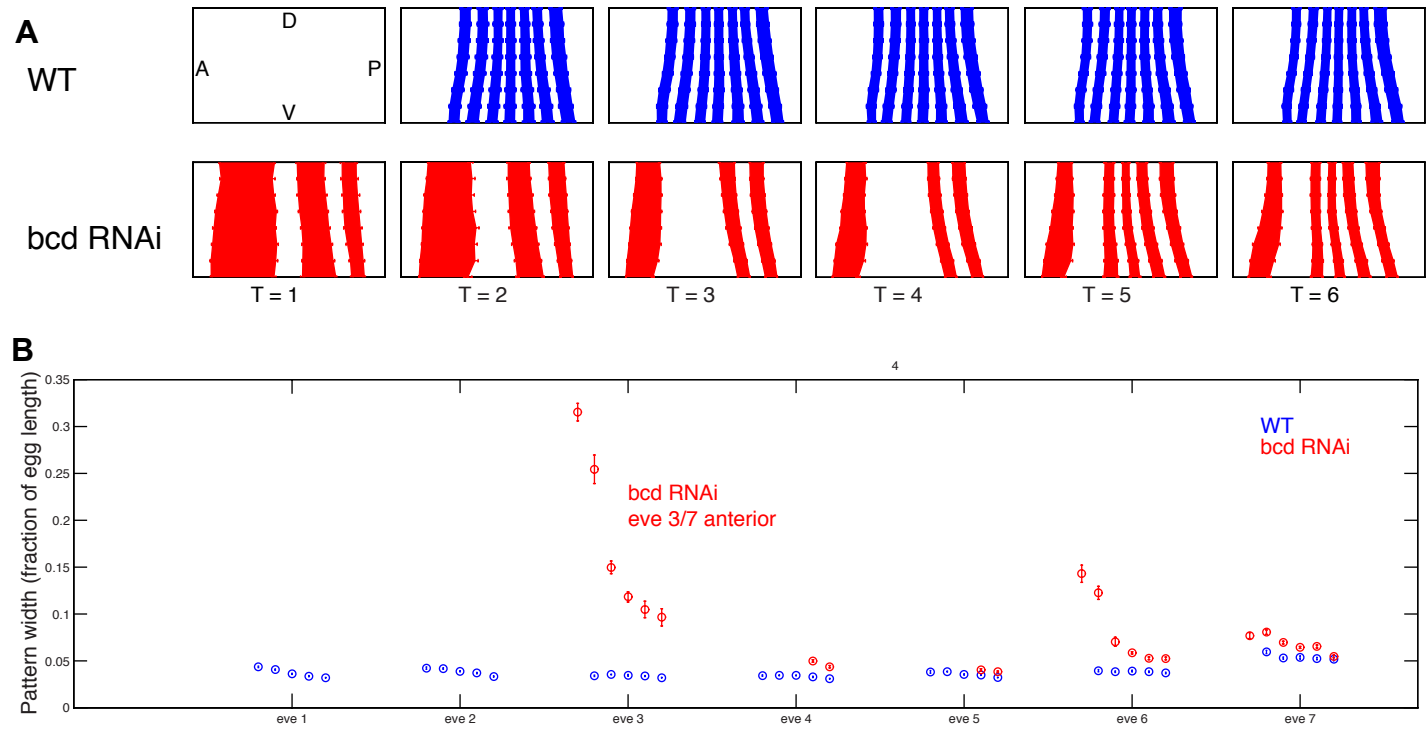


Figure S4:

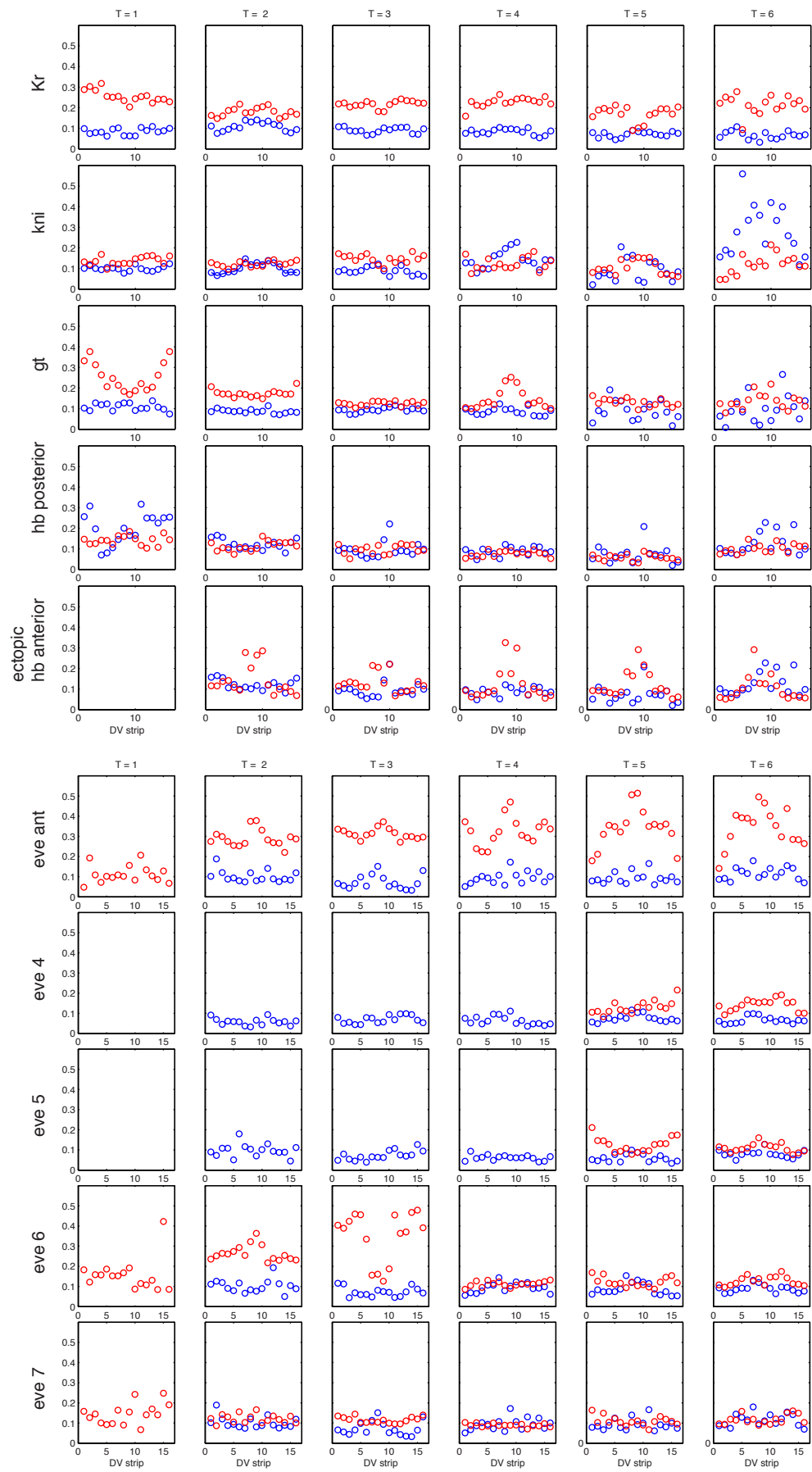


Figure S5:

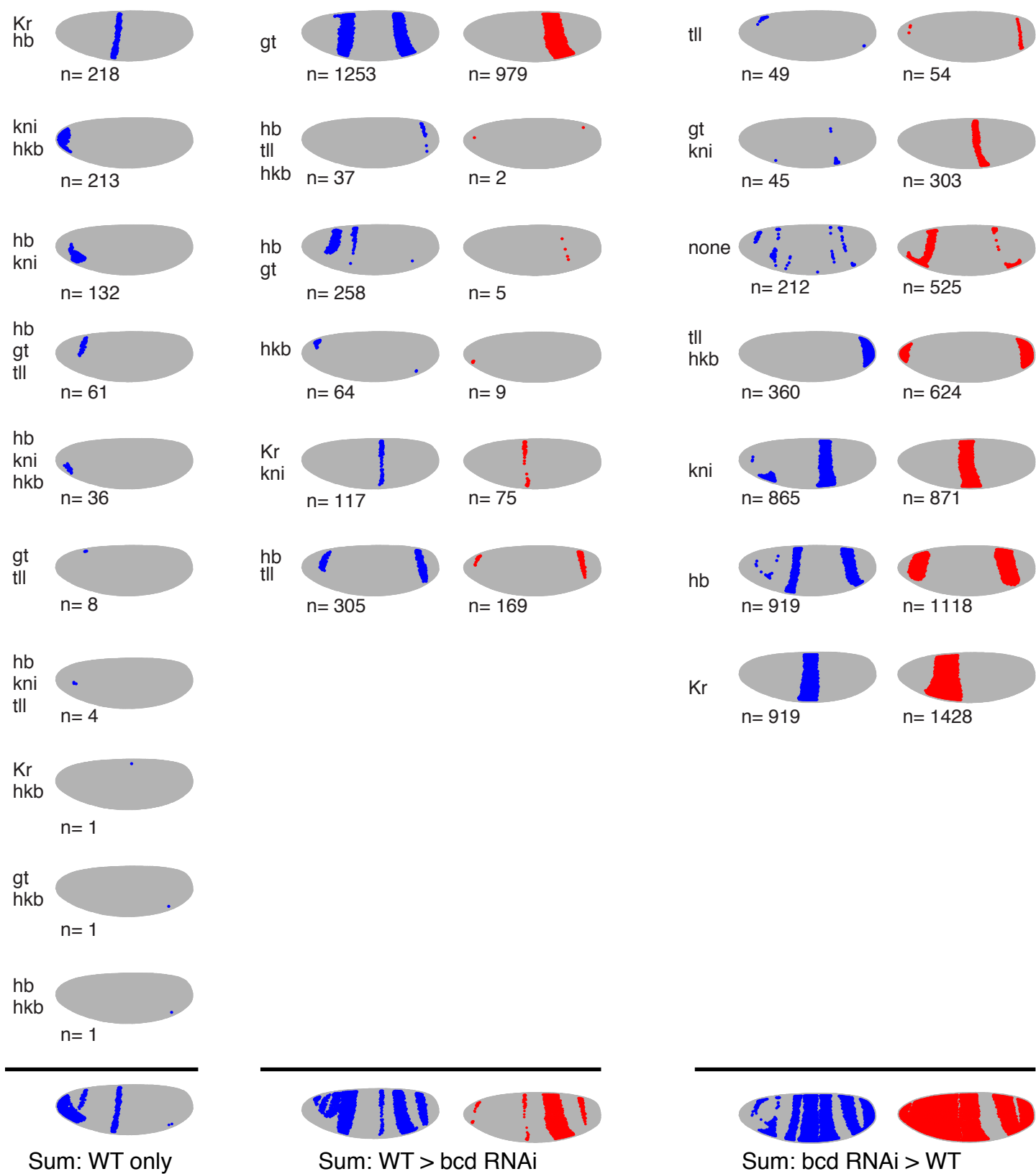


Figure S6

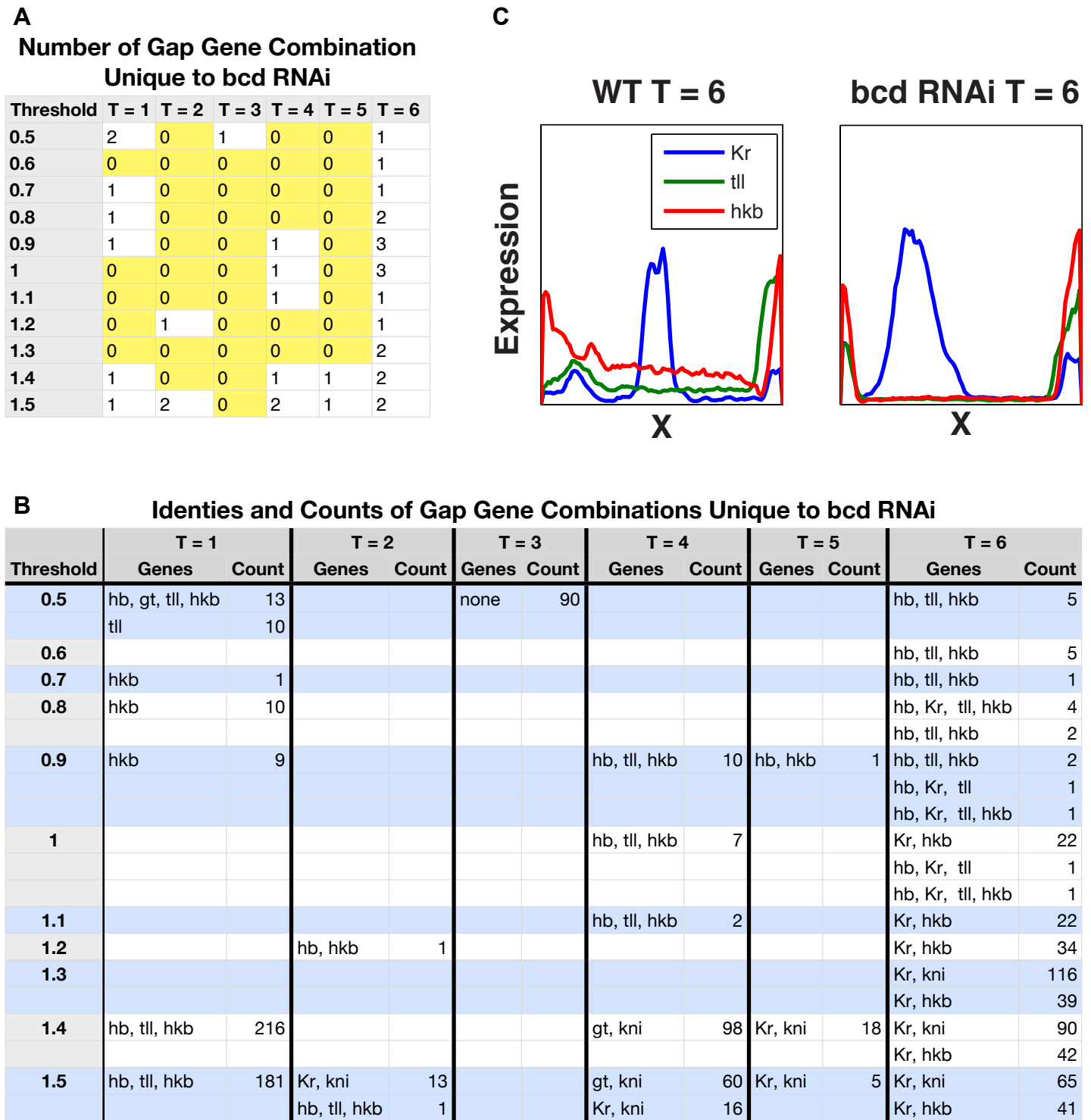


Figure S7

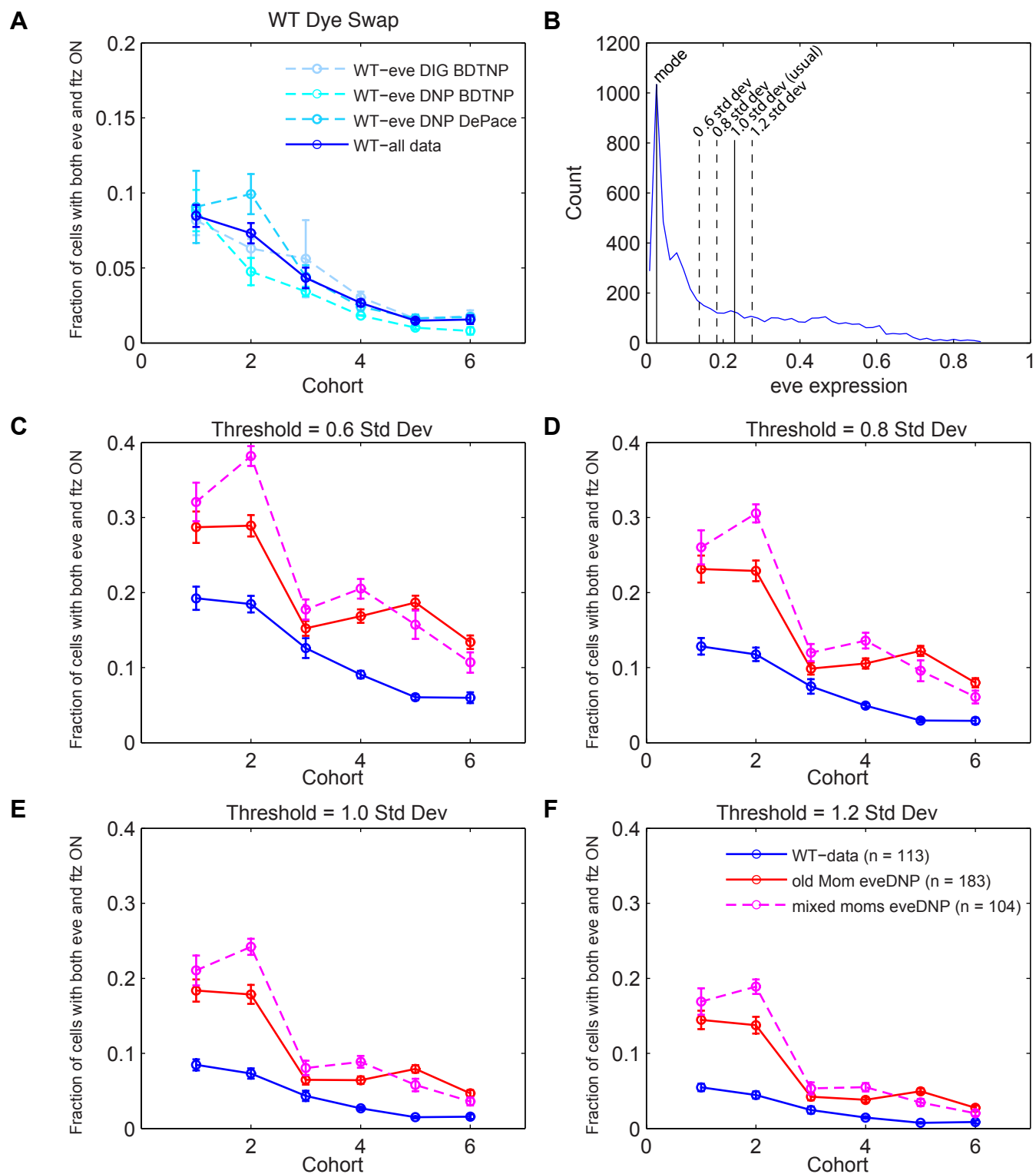


Figure S8

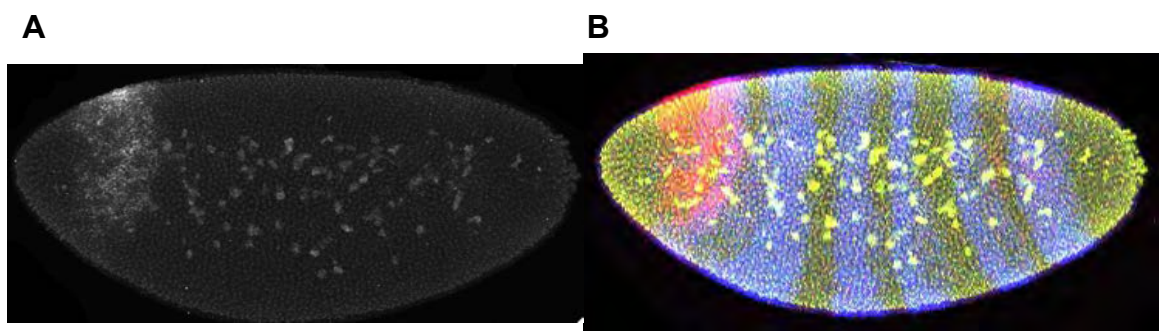


Figure S9

Table S1: Embryos per gene bcd RNAi atlas 20140106

Genes	1	2	3	4	5	6
cad	5	15	24	14	3	6
D	9	19	26	13	5	13
eve	5	10	24	19	28	13
fkh	5	14	16	9	4	7
ftz	38	67	81	24	14	25
gt	25	30	31	13	8	9
hb	16	12	13	17	10	12
hkb	16	11	20	14	6	8
kni	9	15	14	8	6	8
Kr	10	13	16	10	8	10
hb posterior enhancer	6	8	20	9	6	3
gt posterior enhancer	21	18	29	13	6	3
eve3+7 enhancer	3	10	13	7	5	10
eve4+6 enhancer	10	27	26	16	8	8
eve5 enhancer	6	16	32	12	6	9
eve4+6mini enhancer	3	8	12	11	0	3
run	8	20	22	16	9	5
tll	15	20	20	11	6	6
hbP	7	10	14	7	6	9
Average	11.4	18.1	23.8	12.8	7.6	8.8
Sum	217	343	453	243	144	167

**Table S2: Atlas Standard Deviations
20140228**

Gene Name	WT standard deviation	bcd RNAi standard deviation (DNP)
cad	0.165	0.0664
eve	0.129	0.1206
fkf	0.068	0.0268
ftz	0.131	0.1689
gt	0.108	0.0717
hb	0.134	0.0585
hkb	0.106	0.0365
kni	0.099	0.0583
Kr	0.066	0.0813
tlf	0.063	0.0365
D		0.1009
run		0.1509
hbProtein		0.1069
hb posterior enhancer		0.0590
gt posterior enhancer		0.0771
eve3+7 enhancer		0.0741
eve4+6 enhancer		0.0873
eve5 enhancer		0.1247
eve4+6mini enhancer		0.0742

Table S3A: WT thresholds

	T = 1	T = 2	T = 3	T = 4	T =5	T =6
gt	0.35	0.36	0.34	0.27	0.24	0.21
Kr	0.34	0.32	0.28	0.23	0.21	0.20
kni	0.32	0.30	0.31	0.34	0.35	0.32
tll	0.29	0.24	0.19	0.18	0.17	0.16
hkb	0.24	0.30	0.29	0.27	0.23	0.21
hb mRNA	0.41	0.28	0.23	0.23	0.28	0.25
hb Protein	0.21	0.20	0.22	0.22	0.22	0.16

Table S3B: bcd RNAi thresholds

	T = 1	T = 2	T = 3	T = 4	T =5	T =6
gt	0.27	0.30	0.31	0.26	0.22	0.17
Kr	0.24	0.31	0.31	0.32	0.26	0.24
kni	0.20	0.23	0.27	0.29	0.25	0.20
tll	0.26	0.28	0.24	0.19	0.15	0.10
hkb	0.15	0.21	0.24	0.26	0.19	0.18
hb mRNA	0.21	0.26	0.28	0.25	0.22	0.23
hb Protein	0.08	0.13	0.14	0.24	0.27	0.35

Table S4: Enhancer list

Construct	Enhancer sequence	Reference	Primer sequence
eve1	AGGCCATACTTCCCTGAAATGCATAATTGTGCCGCGGCTTTTGATACGCTCCTGGCGGAGAGGGAGATGAGGAAAGGATGCACGGGAACCGCAGCCAAGTGGCAGTTCGAGATTGGCAATCCGCGAGCGGACAATGCCAGAGAATGGGCAACAAGTAGCGGCGAATTAGCAATCCTATCATGCTTTTTATGGCCGCGCAACTCTTGGCCGCGCATCTCAGTTTCATCCGAAGCGGGACCAGGTCCAGGTTCAAGTCGAGGTCCAGTACCCCTGCTATCCCGTCAACCCCTTTAGGGCGATAATCCTTCTAAATGTTTGCATTAAATTCGAGGCGTGGACGGATTAGGGCGTGCTGGCTGGGCGGAACCCGACGACGAAACCGCGAGGACATGCACCCGACTGACCTGCAGCCTACAGATTCTCTGATCTTCGATCTCTAATCCTTTCCGATTGTGCAACTGACTTCTGCACGTGGGTCCGCCCTAATCCTTCCGCCGAGAAGGCGGCAGAGTCCGAGGTAAGTGGGATTATCTGCGATTACCCAGATGATCCGACAGAAATTCGCTGTTTTCAGGGGCTAAATGTCAGCGAAGTCAACTAAATCCAAATCCTTTCCGCCCCCTCTGTTTTATTGTTTGTTCGTTTGTGTTTGAGATTTCTGGCAATTAAAGTTGCCCGTTTTGATGCGCGGGGGCGGGTGCATCAAATCCTTTCCGCGATACCTGTCTGCACAAATGCTGAATTCCGCAATCCATGGATACCCAGATATTTCAGATATCCCAAGGC	Fujioka et al., 1999	AGGCCATACTCACTTCCCTG
eve46	AGGATCCCTGGGCTCTGGGCTCTGGACTATCCGCCGACCCTCCATATCCATGATTTACAATTCTCGTTTTTTTCGCGTTATTTTTTAGGGGCTTTAATGACCGTTCGTAAAGCCGACGAGGACCAGGACCAGGACTCTGCTCACATTTCGCGCAGTCTGATCTAAAAAATGAAATCATTTTTCTTGAAATTTACGGCGCGCTCGAGCAGGACTCTTTGTTCTCGGCCAGGCAATTTGCCTTTTTTCGCTCAGCTCAGTTTTTTTCGTCACGCGGGCATTACCTACACGGCGTTTTATGGCGGAGATGATATTCGCCCTGGGATCGGTTCCGTTTTTAGGCCATAAAAAATAGGCGGCATAAAAAAACTGCAATTGGAATTTCTAGTCTAGTTTTCAAGTTTTTAGGTTTTCCAGGTTTTCTGCCAGCCCGCTAGATTCGCATTTCGCGGAATTCGGAAGCGGAACAGAATGCCAGAATGGTCAGAATCCTGGCTGACCTTGGCTTTTGCCAGGGGGCGTAAAAAAATTGACTCGCTCGGTCGCGGGAATTTTTTAAATCTGACTTTCCAACAATCTCTGATCTGGGTTTGAATGCTAAAAAAAGCAGAACAAAAAGCGGGCATTTTTCTGCGCAATGATCTGTTAATGGGCGGGCTAAAAAACTAAGTCACAAAGTTCACCGGTTGTCGGTTAAATGAAGATGTCTGTTCTACCGAGAAGGATCGAGACATTTCAGCACTTCAAAGCTCCCAAGCGCTCGAAGGATTCGCCGAAGATTCAC	Fujioka et al. 1999	AGGATCCCTGGGCTCTG
eve5	ATATCCCAAGCCGCAAAAGTCAACAGTCCGCAGCAAAATTTCCCTTTTCCGCCGATGTGTTTTTTTTAGCCATAACTCGCTGCAATGTTTGGGCAAGTTTTTCTTCCGCAAAATGGCGAGATGATGCGGGGATATGCGCTGATTGCGTGCAATTATGGACATCTGCGAGGCCCCGAGGAACCTTCTGCTAAATCCTTTATCCGCCCTACAGAACCCCTTTGTGTCCGTTTCGCCGGAGTCCCTTGACGGGTCTTCGACTATTCGCTACAGCAGCTTCCGCTAAAAATTTTCATAACCCACGAGCGGCTCTTCGCCGGAATCCCTGGCAATTACCTTTTTACCTCTTGCCAAATCCGTTGGCTAAAAAACGGCTTCGACTTCGCGTAACGTCTGGACAACAAGACAAAAAGCGCGAAAGGACGGCGGATTCAGGTTAGCATTGCGAATTCGGTCAAACTAAAGGACCGGTTATATAAGGGTTTTATGGCCAGAATCTCTGCATCTCCAGACCCGCAAGAGCTGCGTAAAACTGCAAGCTCTGTTTTGATTCTGCAACTTCAGTTAATGGCCGGATGGCCAGCAATTGCCGGCAATTATAAACAGCGCAGATGTGACTCAGCTTCCATATCTAATCTATATCTCATGCCGAAATCTAGGGTGGGAGCGGAGGGGGCGGGTGCCTGGGTGACTTGCCTGCCAGGGAAAGGGGCGGGGTTACGCGGGTGATAAATGTGCTGATTGGAATGATGCGATCGATTAAAAACCGCAGGGCAATCAATTT	Fujioka et al. 1999	ATATCCCAAGCCGCAAAAG
eve3+7	GGATCCTCGAAATCGAGAGCGACCTCGCTGCATTAGAAAACTAGATCAGTTTTTGTTTTTGGCCGACCGATTTTTGTGCCCGGTGCTCTCTTTACGGTTTTATGGCCCGGTTCCCATTTCCAGCTCTTTGTTCCGGGCTCAGAAATCTGTATGGAATTATGGTATATGCAGATTTTATGGGTCCCGCGCATCCGGTTCGCGGAACGGGAGTCTCGCCGAGAGGTCTCGCCCGGATCCCTTGCCCGGATTCGTTGCGCCGATTAGGAAAGTAGATCACGTTTTTTGTTCCTCATGTGCGCTTTTTTTCGCTGCGCTAGTTTTTTTTCCCGCAACCCAGCGAACTGCCTAATTTTTTAATCTCTACGCGCTTTTCATTGGGCTCCTGGAAAAACCGCGACAAGGTTATAACGCTCTACTTACCTGCAATTGTGGCCATAACTCGCACTGCTCTCGTTTTTAAGATCCGTTTGTGTGTTTTGTGTGCCGATGGCATTACGTTTTTACGAGCTC	Small et al., 1996	GGATCCTCGAAATCGAGAGC
eve2	GGTTACCCGGTACTGCATAACAATGGAACCCGAACCGTAACCTGGGACAGATCGAAAAAGCTGGCCTGGTTTTCTCGCTGTGTGTGCCGTGTTAATCCGTTTGCCATCAGCGAGATTATTAGTCAATTCGAGTTGCAGGCTTTTCGCTTTCCGCTCGTTTTCACATTCGAGTTAGACTTTTATGCGCATCTTGAAACAATCGCGCAGGTTTGGTAACACGCTGTGCCATACCTTCATTAGACGGAATCGAGGGACCCCTGGACTATAATCGCACACAGGAGACCGGGTTGCGAAGTCAGGCGATTCGCGCATCTGCTGCATCTCTCGCGGCGTTTGTGTTTTGTTGCTGGGATTAGCCAAAGGGCTTGACTTGAATCCAATCCGATCCCTAGCCCGATCCCAATCCCAATCCCAATCCCTGTGCTTTTTCATTAGAAAGTCATAAAAAACACATAAATATGATGTCGAAGGGATTAGGGG	Small et al., 1991	GGTTACCCGGTACTGCAATAAC
eve46mini	TCGAGCAGGACTCTTTGTTCTCGGCCAGGCAATTGTCTTTTTTGCCTCAGCTCAGTTTTTTCGTCACGCGGGCATTACCTACACGGCGTTTTATGGCGGAAGTATGATATCGCTGCGGATCGGTTCCGTTTTTAGGCCATAAAAAATAGGCGGCATAAAAAAACTGCAATTGGAATTTCTAGTTCTAGTTTCAAGTTTTTAGGTTTCCAGGTTTTCTGCCAGCCCGCTCGCAATTCGCGGAATTCGGAAGCGGAACAGAATGCCGAATGGTCAGATCCCTGGCTGACCTTGCCTTTTTGCCAGGGGCGGTAAAAAAATTGACTCGCTGCGGTGCGCGGAATATTTTTAAATCTGACTTTCCAACAATCTCTGATCTGGGTT	Fujioka et al., 1999	TCGAGCAGGACTCTTTGTTCTC
hb posterior	TAGCACGAAAAACCGAAGGATTAAAAAAGGAAACTAGACGAGAGTCCCGGGGCGAGGCGAATAGTTGCTCTAATTTTCATTGTCCGCCTTAATGGTTACGCGTAAAAATTTGGCTATCGCGCCAAAAAATAGTGCAGAGACGAGCGGACGCGCAGGACCAATCGTGTGGGATTTCAGTCGACACGCCACGAGATTTATGAAGGCAACTCGCTTTGCATGTTATTCATAGATTTCGCTTCGGTCCCGGTTGTTTTGGTCAGGTAAGACCTTCGATTAAACAATGAAAGTAGTGAATAATCGCGAGCAACACACAAGATACAATATCTATGAGTCTAATGATCATTAGAGCGGTGCGCTCTACATACAATTTGACGACGCTCTGTTTGAAGCCTAAAAACGTCGCAAAAAACACACTTCGCGTAAGACATCCCATTTCTGTGGTCCGATCGTAAAAATTTAGTTTTTATGACCAACGGTGCAGGCGAGTGTGCTGGCTGCCGTTTTTGTGCGCGACCTCAACCCCTTCACCCCATTAAGAAAAAATCGCATCCTGTGAGTGTCTTGCCCGTTTTCCCTCGAAACGGCCACAATTTGTGTCTTTGCGTTTTCTCCTCTCTTTTTGTTTCCACCTAATGTCCGCGCTCAATTGTCTCTTTATGACGCGCTCGGTTGTCTTTTTATGGTGTCTTTGTCTTTGAGCCTCGTTGCACGCGCAAAATCCCTACTTCCTCAACCCCTTTGGCGGACGAGAAAAAGTTGTAGGAGGAGAACGGTTAAGCGAAAAACTCCAATGTCACCTTTTACAAGCCGCGATCTTCTTGAATAGTTTTTGGTCAATTAGGCGAAAGGGTTAATTTTCGATTTTGGCTCTCGGTGGGTTTACTGAGTGAATTCAATGGGCTAAGGCGAGTAAAGGGTTATACTGTTTTTACATTTTACTACTTGAAAAAATACTGAAGAACTTGTAGGAAAAATTTCCAGCACTTTTAAAAAGCATATATAACTTTATGAATATGAACCTCAAAATGTAACCACTGAAAGTGACATGATTTTAAAGTCCCTGAAATGATCATGTCTAAAAATTTCTTTTTTAAATAATTTTAAAAATATTTTTTATAGCATACGAAGTATTTAAAAATGTGAACAGATTAAACACATAAATTTAAAAAGTAAATACAACAGATTAGCATAGAAATAAAAAATCATTTTAAATGTTCCGTCCATAAGTAACGGTCTGGGAAAAATCTTGAAAAATCCCAAAATATATTCGATCCCTTTGGCGGAACATTTGGTGCGATTACATTCGTAATTCGCTGGAATAAGGCACTAAGTCGCCAGCGAAATGAATTCGGACATTGGGCATTGGACAAATGTAAAAAGGACTCTAGAGCCCGACCATTGCAATGGTCCATTGTTGAGCGTCCGAAAGATCTGAAAAACCAACCCCAACCAATCCCGAGCTTAGGCAATCGGCATTGGGAAATAAGCGCAAATATTCTACCCCCCACTCCAAAAGGAGCATT	Wunderlich et al., 2012	TCGAGCAGGACTCTTTGTTCTC
gt posterior	AATTTTATTACCGAAACTTACCATCACTTCGAGATGAAAGTGCAGGAGAGCGCTGATCTTTGCTTATCCCAAACTAGGGGTTTTTATAGGGGTAAAGCGGGAATACAGATTCTCAGAAAAAAATGGCAGTCCATATATGTGATAAGTTGCTTTCAACTTACGTTTACGACAGGGGCCATTTCGAAGCTAAGGATTCGAATATGCGACTGTAAACCATTAAGACAAAGGGCCGCGAAAGGAGTAAACGGCAATTCGCCGAAACCAAGCACGAATCCAAATCCAAACCACTCCTCACCCCTTTTTTGGACGGGTGGGACGGGTCAATATAAGGCAAAACCCCTTTTCAGTCAATATGTCGCTGATTTTCTTTGTACCTTAGCAGCGGACCAATATAAAAAATCGCAGCCACAATGGTCCGAGGAGAGAACCCCTTTTTTTTTAAGGACCGCCGGTGTCCGAAATATCAGTTTATGGCTCTTAAAAAAGCTGCAGCGGTTTTTAGGGCCCGCGGACTCGGAATGAGGCCCTTTTCGACGGAAGCGTCCATTATGCAATAAAAACTTTGGATGTTTTTATAGGCAACGCATAACAGCCTAATACAGCGCATACCGTGGGTGGGCAACCCGTTTCGGCCATCAGGTAGATGATTCCGTAAAAAACGCGCCCAAGGTCAACAACCTAAAGGATTGCAAAATTTGCATCTTTGGTTCCGAGCTCATTATGGCGAAGGAACAGCAGTCCGAGTATATGAATATATGTTTTTATGGCCTGTGTTTTTACGTTTTTTTTTCGCTGAATCTGGTTTTTACACCGAACTTGGAACCTTTTGGCTTCGACTTCGCGCCGCACTTTTTATGGCATCTGCTCGGATCGATTGGGCTGAGTATGATGTCAGGATCTGGAAGGGCTCGGTTTTGGGCCCCGTAACCGCAGGTAAGGGATCGACTCAATGACGGGACGTGACGGTCCGAGTTTTAGTTTTAATCCACCATTTTACGTTTGGATTAGCGCACGGATTAGCGGATAAGTTCGCGGTTTTTACTGATTACCATCGATCGATCCCTCTTTTTATTGCAATTACCCCAATCCCCCTGAATGGCGAGTTGTAA	Wunderlich et al., 2013	
eve late seven stripe	-6.4kb (Ndel) to -4.8kb	Fujioka et al., 1996	-6.4kb (Ndel) to -4.8kb

1 Supplemental Note 1: Patterning by an exponentially distributed morphogen

The following model is reproduced from Eldar and Barkai 2003 and chapter 8 in Alon 2007. It assumes that an exponentially distributed morphogen is the only regulator setting the position of a boundary. In the *D.melanogaster* embryo, maternally deposited *bcd* mRNA is translated to form an exponentially distributed protein gradient (Gregor et al. 2007). In WT, the anterior boundary of *Kr* sits at 44% of egg length from the anterior and shifts to 27% of egg length from the anterior in *bcd* RNAi. The anterior boundary of *kni* shifts from 59% egg length in WT to 46% egg length in *bcd* RNAi embryos. There is evidence that Bcd protein activates *Kr* and *kni*, and may set the anterior positions of these boundaries.

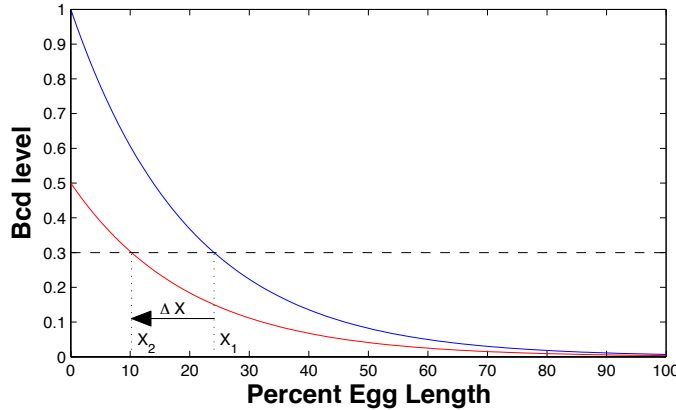


Figure 1: An exponentially distributed morphogen patterns a boundary. The WT gradient is in blue and the perturbed (RNAi) gradient is in red. In this figure, $\beta_1 = 1$, $\beta_2 = 0.5$, and $\alpha = 0.05$.

We begin with an exponentially distributed morphogen $bcd_{WT}(X)$.

$$bcd_{WT}(X) = \beta_1 e^{-\alpha X} \quad (1)$$

$$bcd_{RNAi}(X) = \beta_2 e^{-\alpha X} \quad (2)$$

In this example, the boundary of interest is set at the threshold γ . The RNAi perturbation only changes the input level of the morphogen from β_1 to β_2 . The length scale of the gradient, α is unchanged. It remains unclear if the *bcd* gradient forms through passive diffusion or through a combination of active transport and diffusion, but for this analysis, all that matters is that the shape of the gradient be exponential and that the perturbation does not change the length scale. This model further assumes that the gradient will be

exponential regardless of the total input level, an assumption supported by experimental data (Liu et al. 2013).

X_1 is the location of the pattern boundary in WT and X_2 is the location of the pattern boundary in the perturbed embryo.

$$\gamma = \beta_1 e^{-\alpha X_1} \quad (3)$$

$$\gamma = \beta_2 e^{-\alpha X_2} \quad (4)$$

The shift in boundary position is:

$$\Delta X = X_1 - X_2 \quad (5)$$

To solve for ΔX we set equations (3) and (4) equal to each other.

$$\gamma = \beta_1 e^{-\alpha X_1} = \beta_2 e^{-\alpha X_2} \quad (6)$$

$$\log(\beta_1 e^{-\alpha X_1}) = \log(\beta_2 e^{-\alpha X_2}) \quad (7)$$

$$\log(\beta_1) - \alpha X_1 = \log(\beta_2) - \alpha X_2 \quad (8)$$

$$\log(\beta_1) - \log(\beta_2) = \alpha X_1 - \alpha X_2 = \alpha(X_1 - X_2) \quad (9)$$

$$\frac{\log(\beta_1) - \log(\beta_2)}{\alpha} = X_1 - X_2 \quad (10)$$

$$\Delta X = \frac{1}{\alpha} \log\left(\frac{\beta_1}{\beta_2}\right) \quad (11)$$

Importantly, the shift in the boundary, ΔX is independent of the initial position, X_1 , and depends only on the relative change in input level of the morphogen, $\frac{\beta_1}{\beta_2}$. It follows that all boundaries set by this morphogen will shift by the same amount, ΔX . If this simple patterning mechanism was responsible for setting the boundaries of *Kr* and *kni*, the boundaries of these genes would both shift by the same amount. If Bcd were setting both the anterior and posterior boundary of a pattern, that pattern would shift, but not expand. In reality, the anterior *Kr* boundary shifts by 17% egg length and the *kni* boundary shifts by 13% egg length. The fact that each boundary shifts by a different amount implies other regulators help set these boundaries (see Discussion).